Platform AL: Calcium Fluxes, Sparks, and Waves

1905-Plat

Remodeling of Calcium Handling in Rat Left Atrial Myocytes in a Surgical Model of Elevated Afterload

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Remodeling of the atrial myocardium in heart disease is suggested to increase the risk of atrial fibrillation (AF). Abnormal Ca^{2+} handling is also thought to play a key role in the genesis of AF. In the present study we investigated Ca^{2+} handling in left atrial myocytes from a rat model of elevated afterload produced by surgical banding of the ascending aorta (AoB), which was compared with sham-operated controls (Sham). Cells were isolated at 20 weeks following surgery, corresponding to a pre-heart failure stage of remodeling. Isolated cells were either stained with the lipophilic dye di-8-ANEPPS, or loaded with the Ca^{2+}-fluophore, fluo-3, and imaged using laser scanning confocal microscopy. Measurements of cell volume and surface area from di-8-ANEPPS-stained cells demonstrated that cells from AoB animals were hypertrophied in comparison with cells from Sham controls. Fluo-3-loaded cells were superfused with Tyrode's solution (~22°C), stimulated via field electrodes and changes in $[Ca^{2+}]_i$ monitored using line-scan imaging. The amplitude of the electrically stimulated Ca^{2+} transient was greater in AoB than in Sham cells. Rapid application of caffeine (10 mM) was used to deplete sarcoplasmic reticulum (SR) Ca^{2+}: The amplitude of the caffeine-induced Ca^{2+} transient was greater in AoB than in Sham cells, consistent with a larger SR Ca^{2+} content in AoB than in Sham cells. The incidence and frequency of spontaneous Ca^{2+} transients following a burst of pacing at 4 Hz was greater in AoB cells than in Sham cells. In summary, our data demonstrate for the first time remodeling of atrial myocyte Ca^{2+} handling in a pre-heart failure model of elevated afterload. Changes in atrial Ca^{2+} handling in response to elevated afterload may contribute to increased susceptibility to AF in hypertension.

Supported by the BHF (PG/07/101).

1906-Plat

The Golgi Apparatus is a Juxtanuclear Ca^{2+} Store Regulated by β1-Adrenergic Signalling via Both PKA and EPAC Pathways

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In adult cardiac cells, the Golgi apparatus (GA) was identified as the source of prolonged Ca^{2+} signals that originate from the nuclear 'pole' regions and invade the nucleoplasm. Golgi Ca^{2+} release (GCR) was facilitated by β_1-adrenoceptor activation although the response was blunted markedly by local phosphodiesterase (PDE)-3 and PDE-4 activity, reflecting functional compartmentalization of cAMP signalling. Against a background of subthreshold β_1-adrenoceptor stimulation (2 nM isoproterenol), but not in its absence, the selective Epac activator 8-CPT (10 μM) potently stimulated GCR via a CaMKII-dependent pathway. Once initiated, GCR was entirely unaffected by whole cell Ca^{2+} transients (i.e. spontaneous Ca^{2+} waves or triggered Ca^{2+} release) originating from the sarcoplasmic reticulum (SR). However, GCR was selectively blocked by the GA disrupting agent illimaquinone, while SR function (i.e. total SR Ca^{2+} content, Ca^{2+} sparks and waves) was unaffected. GCR was also blocked by pre-treatment with ryanodine (10 μM), suggesting that Ca^{2+} release from the GA is mediated by ryanodine receptors. These data suggest that in adult ventricular myocytes, the GA can act as a source of nuclear Ca^{2+} that is functionally distinct from the SR and regulated by β_1-adrenergic signalling via both PKA and Epac pathways.

1907-Plat

RyR2(R^{4496C}) Expression Induces Sinoatrial Node Dysfunction

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an arrhythmogenic disease characterized by stress-triggered syncope and sudden death. While ventricular tachycardia appears only during stress conditions, CPVT patients manifest basal sino-atrial node (SAN) dysfunction, but the underlying mechanism remains unknown. We investigated Ca^{2+} handling on SAN from transgenic mice expressing the CPVT ryanodine receptor R4496C mutation ($RyR2^{R4496C}$) and on their wild type (WT) littermates. A 2-fold increase of Ca^{2+} spark frequency in RyR^{R4496C} SAN cells was recorded in basal conditions during the diastolic periods. beta-adrenergic stimulation with 20 nM isoproterenol, multiplied by ~10-fold the occurrence of Ca^{2+} sparks and Ca^{2+} waves. Spontaneous $[Ca^{2+}]_i$ transients recorded by confocal microscope in isolated SAN from $RyR2^{R4496C}$ mice presented a slower beating rate than WT SAN and an impaired positive chronotropic response to beta-adrenergic stimulation. Moreover, isoproterenol induced the appearance of $[Ca^{2+}]_i$ transients and action potentials pauses in 75% of $RyR2^{R4496C}$ SAN cells. Caffeine experiments showed that the sarcoplasmic reticulum (SR) Ca^{2+} load was significantly reduced in the $RyR2^{R4496C}$ SAN cells. *In vivo* telemetric recordings of electrocardiograms (ECG) identified atrial and junctional escape beats overcoming sinus pauses in $RyR2^{R4496C}$ mice following isoproterenol injection consistent with the pauses recorded in isolated SAN. Similar ECGs were observed in CPVT patients during exercise testing, validating the animal model. We conclude that the increased activity of the $RyR2^{R4496C}$ SAN cells during diastolic periods unloads the SR with Ca^{2+}, participating in SAN dysfunction in CPVT.

1908-Plat

Stretch-Dependent ROS Production and Ca^{2+} Signaling in Single Cardiomyocytes

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We have developed a novel technique to robustly attach and stretch single, intact cardiomyocytes using a biological adhesive, MyoTak. Utilizing this system, we have examined the mechanisms by which ''stretch-activated Ca2+ sparks'' (Iribe et. al., 2009) are produced. Stretching a single myocyte by a "physiological" amount leads to a rapid (~100ms) but transient increase in diastolic Ca2+ spark rate. Additionally, stretch can produce arrhythmogenic Ca2+ waves, particularly under conditions of Ca2+ overload. Stretch-dependent sparks and waves are augmented by low concentrations of caffeine, suggesting that stretch-dependent increases in Ca2+ release are due to increased sensitivity of the ryanodine receptors (RyR2) to [Ca2+]i. We previously reported that de-polymerization of microtubules by colchicine application blocked the stretch-dependent Ca2+ spark burst. Here we show that either colchicine or the anti-oxidant n-acetyl cysteine (NAC) abrogate stretch-activated sparks. This suggests that the stretch-dependent pathway depends not only on microtubules but also on the generation of reactive oxygen species (ROS). Using dichlorofluorescein (DCF) to detect cytosolic ROS, we show that stretch triggers an increase in ROS production within seconds, which then returns to baseline levels upon release of stretch. Additionally, either colchicine or diphenyleneiodonium (DPI), an NADPH oxidase (NOX) inhibitor, prevent stretch-dependent ROS production, implicating NOX as a source of stretch-dependent ROS. In summary, our findings suggest that stretch rapidly triggers ROS production via a microtubule and NOX-dependent pathway, oxidizing RyR2s and leading to a burst of Ca2+ sparks and increased probability of Ca2+ waves. The roles played by microtubules and NOX suggest that this stretch-dependent signaling contributes to the physiological regulation of SR Ca2+ release. Our discovery of the link between stretch and the initiation of arrhythmogenic Ca2+ waves suggests that it may also contribute to disease progression and thus form a novel therapeutic target.

1909-Plat

Small Ca^{2+} Release Events in Rabbit Ventricular Myocytes

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The cell-wide Ca^{2+} release that occurs during excitation-contraction coupling in heart is thought to arise from the synchronized activation of many elementary calcium release events, Ca^{2+} sparks. Using cytosolic Ca^{2+} sensitive fluorescent indicators like fluo-4, Ca^{2+} sparks have been visualized in diverse tissues (muscles, neurons and even non-excitable cells) and species (rat, mouse, rabbit, dog, cat and human). During the time when Ca^{2+} is being released from the sarcoplasmic reticulum (SR) in heart during a single Ca^{2+} sparks, a reciprocal Ca^{2+} depletion is occurring in the SR and this can be visualized with low affinity Ca^{2+} indicators like fluo-5N loaded into the SR. Such events, known as Ca^{2+} blinks, can be measured and they reveal the depletion signal with high temporal (milliseconds) and spatial resolution (Brochet *et al.*, PNAS, 2005) and thus reveal information on the inner workings of the SR. The SR organelle must be in excellent optical focus when Ca^{2+} sparks and Ca^{2+} blinks are obtained simultaneously and has enabled us to visualize a population of Ca^{2+} release events that are smaller than Ca^{2+} sparks. These "sub-spark" Ca^{2+} release events have been named 'quarky' Ca^{2+} release events. Here we